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(54) HGF-CONTAINING PHARMACEUTICAL PREPARATION

(57)Abstract:

PURPOSE: To obtain a medicinal pharmaceutical preparation capable of carrying out prolongation of acting time of HGF(hepatocyte growth factor).

CONSTITUTION: The pharmaceutical preparation contains HGF and heparin. Since the HGF-containing pharmaceutical preparation which is effective and long acting at low dosage can reduce administration frequency and amount, relief from patient's pain and reduction of medical expenses can be carried out.

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CLAIMS

[Claim(s)]
[Claim 1] Remedy pharmaceutical preparation characterized by containing HGF and heparin.
[Claim 2] Remedy pharmaceutical preparation according to claim 1 whose HGF is the tissue or the constituent-of-blood origin of Homo sapiens or an animal
[Claim 3] Remedy pharmaceutical preparation according to claim 1 which HGF manufactures by transgenics.
[Claim 4] Remedy pharmaceutical preparation given in either of claims 1-4 which mix HGF and heparin and are prepared at the time of an activity.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the remedy pharmaceutical preparation which can plan durability of the reaction time of HGF in a detail more about the remedy pharmaceutical preparation containing HGF (Hepatocyto Growth Factor, hepatocyte growth factor).
[0002]
[Description of the Prior Art] HGF is bioactive peptide which was discovered by Nakamura and others and which has the most powerful growth acceleration activity to mature hepatocyte. (For example, reference, such as Biochem Biophys.Res Commun., 122, 1450, 1984, FEBS Letter, 22, 311, and 1987), Mass production became possible by the Toshio Kon object engineering technique (for example) Reference, such as Nature, 342, 440, 1989, Proc.Natl.Acad.Sci.USA, 87, 3200, 1990, Biochem.Biophys.Res.Commun., 172, 321, and 1990. The application to a side-effect inhibitor, a wound healing agent, etc. of an anticancer agent is expected as a therapy and preventive. [as opposed to hepatitis, not only liver cirrhosis but a nephritis, cancer, etc. in this factor]
[0003]
[Problem(s) to be Solved by the Invention] As mentioned above, although HGF was matter with which the utilization as drugs is expected, it had become a serious failure when developing this factor as drugs to disappear out of blood in several half-life minutes, even if it prescribes this factor for the patient independently. Incidentally, it is also already known that the main organs in connection with the path clearance from the inside of the blood of this factor are liver. Although this factor is a giant-molecule peptide, for example, a patient will be medicated as injections, if the short drugs of a half-life remain as it is, they are obliged to a frequent administration or self-sustaining administration. Therefore, a patient was forced a bigger pain in a therapy compared with lasting long drugs. Moreover, if the path clearance out of blood is quick, while the medication of a large quantity will be needed and inviting the jump of a health care cost, it is necessary for a large quantity to produce this factor. Although the attempt which is going to conquer this point with a general pharmaceutical preparation technique also occurs, it was not designed corresponding to the property of this factor, and sufficient effectiveness is not acquired. Made that this invention should solve the above-mentioned technical problem, maintaining the bioactive of HGF, the object of this invention reduces the path clearance out of blood, and is to offer the pharmaceutical preparation which can make the duration of action of this factor extend.
[0004]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, when this invention persons examined making the reaction time of HGF maintain wholeheartedly, they found out that the path clearance of HGF from the inside of blood might be reduced using HGF and heparin together in consideration of the property of the heparin compatibility which HGF has, i.e , by making the complex of HGF and heparin form. Moreover, formation of the complex concerned also checked not giving trouble to the

manifestation of the bioactive which HGF has. This invention is made based on this knowledge. That is, the remedy pharmaceutical preparation of this invention consists of containing HGF and heparin

[0005] If HGF which is the active principle of this invention which consists of the above-mentioned configuration is refined by extent which can be used as a remedy, what was prepared by various approaches can be used for it. As the preparation approach of HGF, various kinds of approaches are learned, for example, it can extract and refine and can obtain from blood cells, such as organs, such as the liver of mammalians, such as a rat, a cow, a horse, and a sheep, a spleen, a lung, bone marrow, a brain, the kidney, and a placenta, a platelet, and a leucocyte, plasma, a blood serum, etc. Moreover, the primary culture cell and established cell line which produce HGF can be cultivated, separation purification can be carried out from cultures (a culture supernatant, cultured cell, etc.), and HGF can also be obtained. Or recombination HGF which inserts in a suitable vector the gene which carries out the code of HGF by the gene engineering-technique, inserts a nest and this in a suitable host, and carries out a transformation and which is made into the object from the culture of this transformant can be obtained (for example, reference, such as Nature, 342, 440, and 1989). Especially the above-mentioned host cell is not limited, but can use various kinds of host cells used by the gene engineering-technique from the former, for example, Escherichia coli, a Bacillus subtilis, yeast, mold, vegetation, or an animal cell.

[0006] As an approach of carrying out extract purification of HGF from a body tissue, a carbon tetrachloride can be injected intraperitoneally to a rat, the liver of the rat changed into the hepatitis condition can be extracted and ground, and, more specifically, it can refine in the usual protein purification methods, such as gel column chromatographies, such as S-sepharose and heparin sepharose, and HPLC, for example. Moreover, using the modifying-gene method, by the expression vector which included the gene which carries out the code of Homo sapiens's HGF amino acid sequence in vectors, such as a bovine papilloma virus DNA, the transformation of an animal cell, for example, a Chinese hamster ovary cell (CHO) cell, mouse C127 cell, the ape COS cell, etc. can be carried out, and it can obtain from the culture supernatant.

[0007] that, and a part of other amino acid sequences are inserted, or 1 or two or more amino acid have combined with the amino terminal and/or the C terminal HGF obtained in this way **** -- or a sugar chain -- the same -- deletion -- or you may permute. [that a part of the amino acid sequence is permuted by deletion or other amino acid] As this HGF said ****, the matter of a publication is mentioned to JP,3-130091,A, the international disclosure WO 90/No. 10651 official report, etc., and these can also be applied to this invention and contained in the range of this invention, for example.

[0008] If refined as heparin which is other components of this invention by extent which can be used as a remedy, anything can be used and especially the origins (for example, a cow, Buta, etc.) will not be limited, either. Moreover, especially the molecular weight of the heparin used is not limited, either, but both the amount heparin of macromolecules low-molecular-weight heparin and those mixture can be used. As an operating rate of the heparin to HGF, heparin is set to about 0.01-50mg to HGF1pmol. Although it is satisfactory even if it may be unable to discover HGF path clearance lowering effectiveness with the amount sufficient by less than 0.01mg of the heparin used and exceeds 50mg, since effectiveness can be demonstrated in the amount till then, there is little need of adding exceeding the amount.

[0009] the pharmaceutical preparation which the object of this invention prescribes for the patient the pharmaceutical preparation containing HGF and heparin which were prepared beforehand, or contains HGF and heparin -- business -- it is attained by sometimes preparing and prescribing a medicine for the patient. As an application symptom, the therapy and prevention of hepatitis, liver cirrhosis, a nephritis, cancer, etc., side-effect control of an anticancer agent, acceleration of wound healing, etc. are mentioned.

[0010] Although the pharmaceutical preparation of this invention can take various

formulation (for example, liquids and solutions, a solid preparation, a capsule, etc), only HGF and heparin which are generally an active principle are made into injections with the support of them and common use, or let it be a medicine for external application with the support of common use The injections concerned can be prepared with a conventional method, for example, HGF and heparin can be filtered with a filter etc., after dissolving in suitable solvents (for example, sterilized water, the buffer solution, a physiological saline, etc.), and it can sterilize, and they can be prepared by filling up a sterile container subsequently. As an HGF content in injections, it is preferably adjusted to 0.001 to 0.1 (W/V %) extent, and a heparin content is usually suitably adjusted 0 0002 to 0 2 (W/V %) extent according to an HGF content. Moreover, as a medicine for external application, it is pharmaceutical-preparation-ized by the shape of ointment, gel, and which liquefied dosage forms, and the HGF content in pharmaceutical preparation can be suitably adjusted according to the application disease of a medicine for external application, an application site, etc., for example On the occasion of pharmaceutical-preparation-izing, a stabilizing agent is added preferably and albumin, a globulin, gelatin, a mannitol, a glucose, a dextran, ethylene glycol, etc. are mentioned as a stabilizing agent, for example. Furthermore, the pharmaceutical preparation of this invention may contain an additive required for pharmaceutical-preparation-izing, for example, an excipient, the solubilizing agent, the antioxidant, the aponia-ized agent, the isotonizing agent, etc. When it considers as liquid preparations, it is desirable for cryopreservation or freeze drying to remove moisture and to save. lyophilized products -- business -- it is used for it, sometimes adding distilled water for injection etc. and sometimes remelting.

[0011] The pharmaceutical preparation of this invention may be prescribed for the patient according to the suitable route of administration according to the gestalt of this pharmaceutical preparation. For example, it can be made the gestalt of injections and a vein, an artery, hypodermically, intramuscular, etc. can be medicated. Although the dose is suitably adjusted by a patient's symptom, age, weight, etc., it is usually 0.01mg - 100mg as HGF, and it is appropriate for it to prescribe this for the patient in 1 time per thru/or several steps day.

[0012]

[Example] Hereafter, although this invention is explained to a detail based on an example and the example of a trial, this invention is not limited to these examples. In addition, although HGF of Type 1 stated to the total theory (for example, Critical Reviews in Oncogenesis, 3, 27-54, 1992, a metabolic turnover reference, such as 28, 599-608, and 1991) of Nakamura and others was used in the example described below Even if it is already known that HGF of Type 2 and Type 3 also has activity equivalent to HGF of Type 1 and it uses the derivative of each type for Type 2 and type 3 list, it is clear that the same effectiveness is acquired.

[0013] They are HGF1mg, heparin 4g, mannitol 1g, and polysorbate 80 in 100ml of example 1 physiological salines The solution containing 10mg was prepared in sterile, 1ml was poured distributively in sterile into each vial bottle, it freeze-dried according to the conventional method, and lyophilized products were obtained.

[0014] To 100ml of 0.02M phosphate buffer solutions of example 20.15M NaCl and pH7.4 which contains polysorbate 80 0.01%, the water solution which added HGF1mg, heparin 4g, and 100mg of human serum albumins was prepared in sterile, 1ml was poured distributively in sterile into each vial bottle, it freeze-dried according to the conventional method, and lyophilized products were obtained.

[0015] Hereafter, this invention is explained based on the example of a trial. In addition, the heparin used for the trial is as follows.

The amount heparin of macromolecules: The product made from a sigma, part number H 7005[sodium salt, grade II, the Buta intestinal-mucosa origin, molecular weight: 25000-35000 (the laser **** method), 18000-23000(gel filtration technique)]

Low-molecular-weight heparin: Sigma company make, part number H 5640 (sodium salt, the Buta intestinal-mucosa origin, molecular weight: 4000-6000)

[0016] Only HGF of the marker concentration (0.8pM) which the radioactivity which appeared in the effluent when flowing in reached comparatively, and carried out the indicator of the HGF-heparin complex to example of trial 1 rat liver by liver extractability 125I And after mixing the amount heparin of giant molecules (0.1mg [ml] /, 1mg [ml] /, and 3mg/ml) with this preparation, respectively, perfusion of each complex which incubated for 50 minutes and was created at the room temperature was carried out once to the rat by passage (a part for perfusion rate and 12ml/). Perfusate used the neutral buffer solution [120mM NaCl, 4 8mM KCl, 1mMKH2PO4, 1 2mM MgSO4, 2.2mM CaCl2, 20mM MES (pH7.4)] containing 20% (V/V) of cow erythrocyte, 2% (W/V) of cow serum albumin (BSA), and 5mM glucose. Time amount transition (drawing 1 left-hand side) and its liver extractability (drawing 1 right-hand side) of the rate of the radioactivity of the TCA-precipitate nature in the hepatic vein to the radioactivity of the trichloroacetic acid (TCA)-precipitate nature in an influent were measured. In addition, liver extractability was calculated from (activity in activity-hepatic vein in influent)/(activity in an influent). [under a steady state] As shown in drawing 1 , when it mixed with heparin 1mg [/ml] or more, liver extractability fell notably. Moreover, it is in when it combines and considers that the main organs in connection with the path clearance out of the blood of HGF are liver. Also on vivo conditions, it was suggested that the path clearance out of the blood of HGF also falls notably by heparin

[0017] Only HGF of the marker concentration (500pM) which carried out the indicator by time amount transition 125I of the TCA precipitate nature radioactivity in plasma when injecting example of trial 2125 I-HGF-heparin complex intravenously With this preparation, respectively And the amount heparin of macromolecules (20mg [ml] /, 40mg [ml] /, and 80mg/ml), Or after mixing low-molecular-weight heparin of 80mg/ml, respectively, about 0.25ml of each complex which incubated for 50 minutes and was created at the room temperature was injected intravenously from the femoral vein to the rat. Subsequently, it collected blood from the femoral artery and transition of the TCA-precipitate nature radiation concentration in plasma was measured. In addition, under these conditions, the doses of 0.13pmol / rat, and heparin are 5mg / rat, 10mg / rat, 20mg / rat, and low-molecular-weight heparin in the amount heparin of macromolecules, respectively, and the dose of HGF is equivalent to 20mg / rat. The obtained result is shown in drawing 2 . In addition, the dose standardized and showed the plasma concentration obtained as a result. As shown in drawing 2 , at the amount heparin of macromolecules, lowering of the HGF path clearance out of blood [Tsuguaki in low molecular weight heparin] was seen by 20mg / rat more than 5mg / rat.

[0018] The rat isolation hepatocyte (2.5x105 cells / ml) prepared by the DNA synthesis acceleration (effect of contact time with HGF) collagenase perfusion method of the primary culture hepatocyte by example of trial 3HGF was put in so that the number of cells per two might become 7x104 pieces to the dish for culture 1cm, and it was cultivated for 24 hours in the Williams' medium E culture medium (1nM insulin, 1nM dexamethasone, 5% (V/V) calf serum, and 30 mg/l kanamycin mono-sulfate are included). It exchanged for the fresh thing of the culture medium same 2 hours after culture initiation the middle. Incubation was continued until it washed the cell, it added MEDIUMU for the said culture and it became 24 hours after culture initiation in a total of 28 hours, after versatility carried out time amount (0.3 to 28 hours) incubation of HGF (0-250pM), while exchanging the culture medium for the Williams' medium E culture medium (1nM insulin, 1nM dexamethasone, the 5U/ml aprotinin, and 30 mg/l kanamycin mono-sulfate are included). The deoxyuridine (extraction concentration, 0.3microcurie [ml] /, 0.14nM) which carried out the label by 125I 22 hours after was added with the non-indicator object (the last concentration, 480nM) in the middle of the incubation. DNA synthesis was evaluated by 28 hours after (6 hours after 125I-deoxyuridine addition) measuring the amount of incorporation of 125I-deoxyuridine. The result was shown in drawing 3 . In addition, the result expressed as 100 the maximum activity acquired when contacting HGF of 90pM for 28 hours. DNA synthesis was increasing, so that from drawing 3 and the contact time of HGF and the hepatocyte which is

a target cell was long. This shows that the direction in which HGF was made to exist over long duration by the inside of blood leads to enhancement of the effectiveness.

[0019] DNA synthesis HGF1nM or 12.5nM(s), and heparin (concentration, zero to 100 mg/ml) of the primary culture hepatocyte by example of trial 4HGF-heparin complex were pre incubated for 50 minutes at the room temperature, and complex was made to form. In addition to the culture hepatocyte in 500micro of culture media I which showed 20micro of this mixed solution I to the example 3 of a trial, it incubated for 28 hours Therefore, as the last concentration in the inside of a culture medium, it added so that it might be set to about 40 pM(s) and 500pM(s) by HGF and might be set to zero to 4 mg/ml by heparin The middle, 22 hours after HGF-heparin mixed liquor addition, 125I-deoxyuridine was added like the example 3 of a trial, and DNA synthesis ability was measured The result is shown in drawing 4 . In addition, in drawing 4 , DNA synthesis ability under heparin nonexistence was set to 100, and it expressed as the rate (%) to it. Bioactive also with complex expensive enough with high-concentration heparin was carried out so that clearly from drawing 4 . The amount of HAPERIN called 10mg / rat sufficient incidentally for the path clearance lowering in blood of HGF shown in the example 2 of a trial corresponds [ml] in about 1mg /, when the volume (about 10ml / rat) of circulating plasma is taken into consideration.

[0020] The time amount transition normal rat and carbon-tetrachloride processing rat (the carbon tetrachloride diluted with olive oil 10 times) of the TCA precipitate nature radioactivity in plasma when injecting heparin intravenously after example of trial 5125 I-HGF intravenous injection 1ml / 100g weight administration being carried out at the rat abdominal cavity, and using the rat of 24 hours after 125I - After injecting intravenously HGF of the tracer scale (0.13pmol / rat) which carried out the indicator from a femoral vein, time amount transition of the TCA-precipitate nature radioactivity in the plasma when injecting the amount heparin of macromolecules intravenously after 11 - 16 minutes with various doses (0, 10, 25 or 50mg / rat) was measured. In addition, blood collecting was performed from the femoral artery. Time amount transition of the TCA precipitate nature radioactivity in the plasma when taking after injecting 125 I-HGF intravenously to a normal rat for 16 minutes, and injecting heparin intravenously to drawing 5 , is shown. Moreover, time amount transition of the TCA precipitate nature radioactivity in the plasma when taking after injecting 125 I-HGF intravenously to a normal rat (control) and a carbon-tetrachloride processing rat for 11 minutes, and injecting heparin (25mg / rat) intravenously to drawing 6 , is shown. As shown in drawing 5 and 6, it is distinct that the plasma concentration of 125 I-HGF rises in passing away by heparin administration. As this reason, both the causes of that the path clearance in blood of HGF falls by heparin and 125 I-HGF combined with each organization chart side being removed by heparin, and shifting into circulation blood can be considered. Here, an important thing is the point that carry out carbon-tetrachloride processing and the effectiveness of the heparin as a normal rat also with the same hepatitis inducement rat is seen.

[0021]

[Effect of the Invention] As explained above, the HGF content drugs which have effective durability by the low dose can be obtained by adding the excipient and stabilizing agent of the various kinds further usually used by mixing HGF with heparin and making complex form. Therefore, according to this invention, since the count of administration and a dose can be reduced, relaxation of a patient's pain, reduction of a health care cost, etc. can be aimed at.

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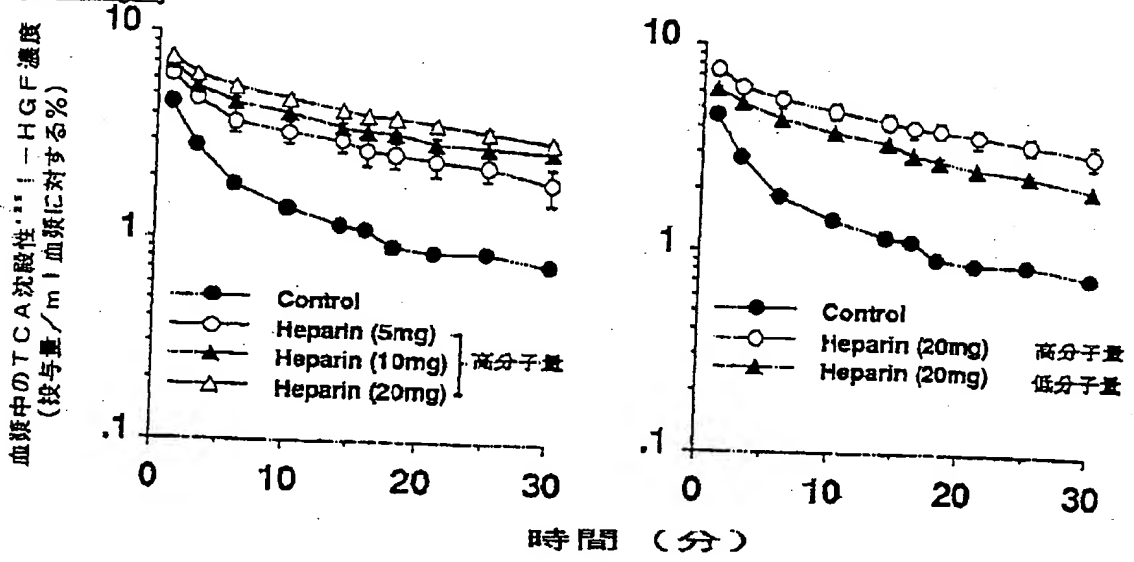
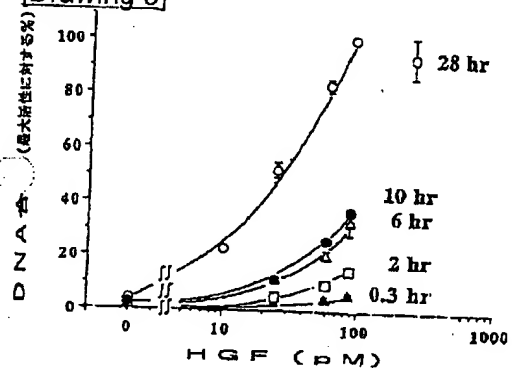
DRAWINGS

[Drawing 2]



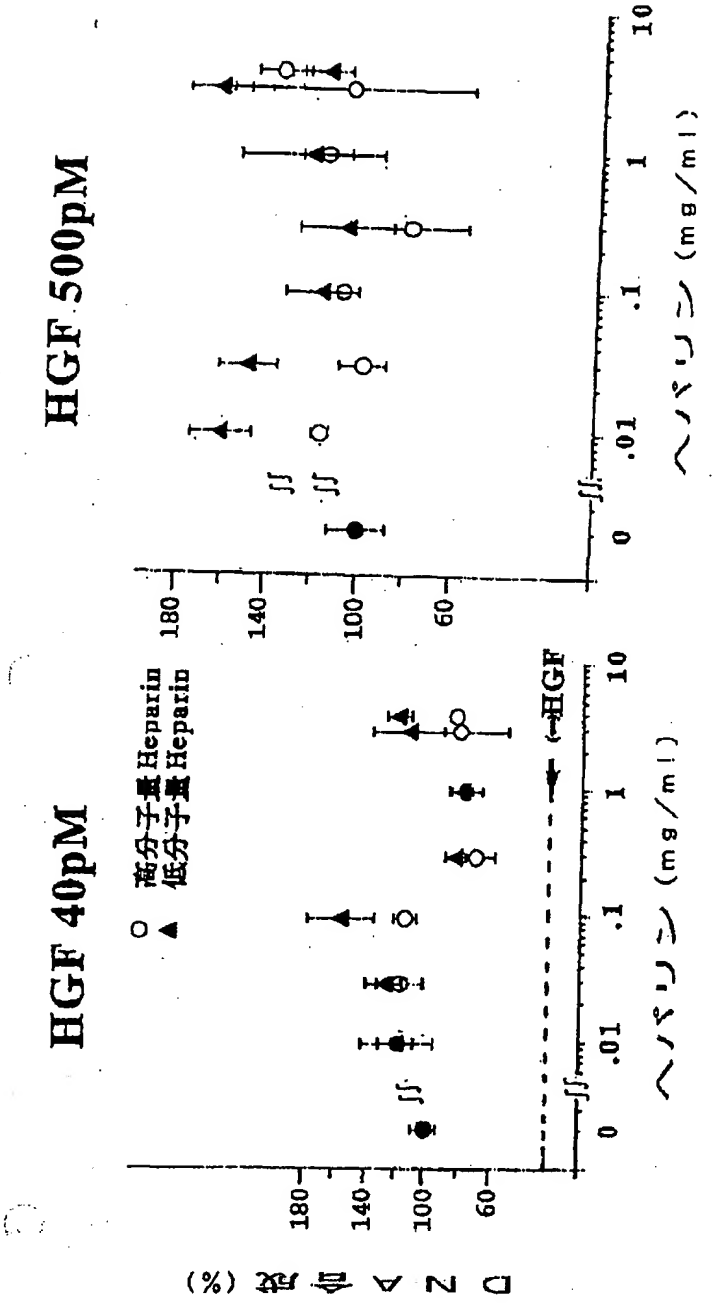
[Drawing 3]



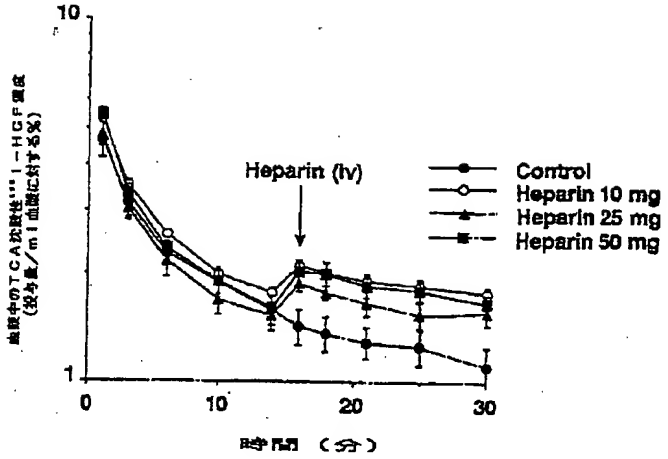
[Drawing 1]

肝抽出率
0.0
0.1
0.2
0.3
0
0.1
1
3
ヘパリン（mg/ml 濃度）
放射活性の割合
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
時間（分）
0.1mg/ml Heparin
1mg/ml Heparin
3mg/ml Heparin
125 I-HGF only

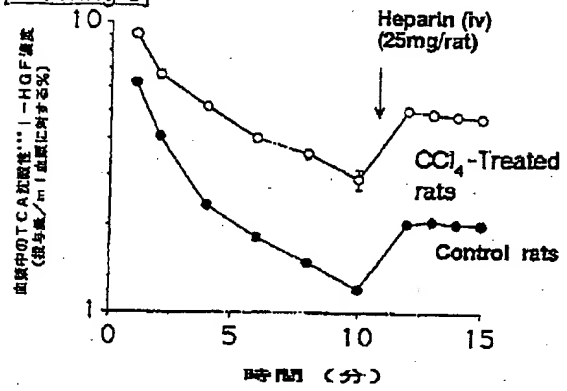
[Drawing 4]


HGF 500pM
HGF 40pM
○ 高分子量 Heparin
▲ 低分子量 Heparin
HGF
DNA合成 (%)
ヘパリン (mg/ml)
180
140
100
60
0
.01
.1
1
10

[Drawing 5]



[Drawing 6]



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